REMARKS

(I) AMENDMENTS AND CLAIM STATUS

Claims 71, 72, 78 and 81 have been amended and claims 73 and 76 have been canceled without prejudice or disclaimer. Claim 87 has been added.

(II) OBJECTIONS TO THE CLAIMS

Claims 71 and 72 have been amended to address the Examiner's objection regarding dependency from canceled claim 67. Claim 76 has been canceled, mooting the objection. Withdrawal of the objections respectfully is requested.

(III) CLAIM REJECTIONS 35 U.S.C. § 112

Claims 60, 73, 76, 78, and 81 are rejected under 35 U.S.C. § 112 as allegedly failing to comply with the written description requirement. Specifically, the Examiner asserts that the originally filed specification fails to provide support for:

> 1) the first and second peptides being selected from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer; 2) separating the bound peptides from unbound peptides to increases the relative concentration of the bound peptides to unbound peptides by at least 100 fold; 3) subjecting the bound peptides to a concentrating step after elution from said antibodies and before introduction into said mass spectrometer.

Applicant respectfully traverses and requests withdrawal of the rejection.

An applicant need not describe exactly the subject matter claimed, but the description must clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed."[1] The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon reasonably conveys to the artisan that the inventor had possession of the invention.[2] Applicant respectfully submits that the specification describes the invention in sufficient detail such that a skilled artisan would

---

[1] *Vas-Cath Inc.* v. *Mahurkar,* 935 F.2d 1555, 1563 (Fed. Cir. 1991).

[2] *See id.* (citing to *Ralston Purina Co. v. Far-Mar-Co, Inc.,* 772 F.2d 1570, 1575,227 USPQ 177, 179 (Fed.Cir.1985) (quoting *In re Kaslow,* 707 F.2d 1366,1375,217 USPQ 1089,1096 (Fed.Cir.1983).

recognize that the inventor fully possessed the claimed subject matter, and requests withdrawal of the rejection.

The specification at page 15, line 9 *et seq.* clearly supports the recitation "the first and second peptides being selected from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer" found in claim 60. Although the Examiner states that "[a]t best, page 15 states that the peptide has a sequence that results from cleavage of the proteolytic enzyme," applicant respectfully submits that is incorrect. At page 15, beginning at line 29, the specification states "the peptide should ionize well by either electrospray (ESI) or matrix-assisted laser desorption (MALDI) ionization." A skilled artisan would understand that this characteristic can be estimated by software programs or determined experimentally by MS analysis of a digest of the protein in question to see which peptides are detected at highest relative abundance. A skilled artisan also would understand that peptides that "ionize well" and "are detected at highest relative abundance" are those that provide a high signal to noise relative to peptides that do not ionize well or are detected at lower relative abundance. See also the appended Supplemental Declaration by Professor Steven Carr ("Carr supplemental declaration") which also concludes that the specification demonstrates that the inventor clearly possessed the subject matter of claim 60.

Applicant pointed to page 30, lines 6-14 for support of claims 78 and 81. Page 30, lines 12-19 recites:

> a series of monitor peptides with their corresponding labeled standards) are introduced into a chromatography column (such as a C18 reverse phase column, forming part of a chromatography system also under computer control) and eluted from this reverse phase column (typically over 5 to 30 minutes) by a gradient (typically of 0-70% acetonitrile in water with 0.05% trifluoroacetatic acid). The output (eluate of the column) is directed into the mass spectrometer, with the result that only one or a few of the monitor peptides appear at any one time, thus allowing the MS to measure each individually without the potential for interference of the other monitor peptides.

A skilled artisan readily would understand that chromatography of peptides on a C18 reverse phase column, such that only one or a few monitor peptides appear at any one time, has the effect of both separating (the primary object of chromatography) and concentrating the peptides upon elution (samples applied to C18 columns almost universally are of larger volume than the volume of separated peptide peaks eluted from the column). Claims 78 and 81 have been amended above to refer to a "chromatography" step rather than a "concentrating" step. For the

reasons set forth above, applicant respectfully submits that the specification provides a written description of the claimed subject matter and withdrawal of the rejection respectfully is requested.

(IV) CLAIM REJECTIONS 35 U.S.C. § 103

Claims 44, 47-61, 64-65, 67 and 71-80 are rejected under 35 U.S.C. § 103 as obvious over Geng *et al.* in view of Little *et al.* Specifically, the Examiner asserts that Geng *et al* teaches substantially all of the elements of claims 44, 47-61, 64-65, 67 and 71-80. Although the Examiner admits that Geng *et al.* fails to teach the use of anti-peptide antibodies specific for a first and second peptide, Little is cited as remedying the admitted deficiencies of Geng. Applicant respectfully traverses.

The Supreme Court addressed the issue of obviousness in *KSR Int'l Co. v. Teleflex Inc.*[3] In *KSR* the Court reiterated that the *Graham* v. *John Deere Co.*[4] factors still control an obviousness inquiry. Those factors are: 1) "the scope and content of the prior art"; 2) the "differences between the prior art and the claims"; 3) "the level of ordinary skill in the pertinent art"; and 4) objective evidence of non-obviousness.[5] The Court in *KSR* emphasized that the Examiner must provide an explanation to support an obviousness rejection.[6] That explanation must include a rational underpinning to support the legal conclusion of obviousness, which cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning.[7]

Applicant respectfully submits that the Office Action fails to properly assess both the scope and content of the prior art and the differences between the prior art and the claims. Accordingly, the Examiner has failed to meet the burden of presenting a *prima facie* case of

---

[3] 127 S. Ct. 1727 (2007).

[4] 383 U.S. 1 (1966).

[5] *KSR Int'l Co.*, 127 S. Ct. at 1734 (quoting *Graham*, 383 U.S. at 17-18).

[6] *KSR Int'l Co.*, 127 S. Ct. at 1741 (stating analysis should be made explicit and citing to In *re Kahn*, 441 F.3d 977 (Fed Cir 200));.

[7] *See, KSR Int'l Co.* 127 S. Ct. at 1741 (discussing obviousness analyses and citing *In re Kahn*, 441 F.3d 977,988 (Fed. Cir. 2006)).

obviousness for claims 44, 47-61, 64-65, 67 and 71-80, and withdrawal of the rejection is requested.

A. Geng Fails to Teach Each Element of the Invention Ascribed to that Reference.

Not only does Geng fail to "teach the use of anti-peptide antibodies specific for the first and second peptides" as the Examiner concedes in the Office Action of December 8, 2008, but also fails to teach other elements of the claims. Specifically, contrary to the Examiner's assertions at pages 6-7 of the Office Action, Geng does not teach at page 299 that "the enormous complexity of the sample produced by proteolysis was reduced by using affinity chromatography methods to select specific peptides." Rather, the text in Geng relied on by the Examiner refers to the use of affinity methods to select peptides "with unique structural features" (Geng p299, 2nd column, line 7). In other words, Geng describes preparing a class of peptides defined by a structural feature, for example a glycan structure containing N-acetyl glucosamine, and does not describe selection of "specific peptides" as stated by the Examiner.

The Examiner also states that Geng teaches the use of a first reagent specific for a first peptide and a second reagent specific for a second peptide (Office Action of Dec. 8, 2008 at page 9) but the Examiner provides no support for the assertion that Geng describes use of two reagents for selecting two analytes in a single step. Rather, Geng describes using an affinity reagent, such as a lectin, which has affinity for carbohydrate structures, to select a group of glycopeptides, followed by subsequent use of reverse phase liquid chromatography to further separate these peptides. Neither the lectin nor the chromatography medium employed in Geng is specific for a single target peptide, and in any case, the sample digest is never contacted to two specific reagents together. Indeed, if Geng were to use two class-specific reagents to select different classes of peptides, and then combine the selected peptide classes for mass spectrometric analysis, this would produce a sample that was more complex than the individual peptide classes! The resulting effect would be to undermine the very purpose for which affinity reagents are used in Geng, namely reducing the complexity of a complex digest.

The accompanying Declaration by Professor Fred Regnier (the "Regnier declaration"), senior author of Geng, further discusses how Geng describes methods of obtaining classes of peptides, and how this differs from the methods recited in the instant claims. See Regnier declaration at paragraphs 8-13. Specifically, Professor Regnier describes that the purpose and

result of using a lectin affinity agent (isolation of a *class* of peptides) is quite different than the result obtained with antibodies that target specific peptide sequences (isolation of specific peptides).

B. Little *et al.*, U.S. 6,207,370, Cannot Make Up for the Deficiencies of Geng *et al.*

The Examiner admits that Geng fails to "teach the use of anti-peptide antibodies specific for the first and second peptides" but asserts that Little compensates for these acknowledged deficiencies. Office Action of December 8, 2008, at page 8. However, for the reasons described below, Little does not teach or suggest contacting **a** sample with a first anti-peptide antibody specific for a first peptide and a second anti-peptide antibody specific for a second peptide, where the second antibody is different from the first antibody.

The passages in Little relied upon by the Examiner do not support the use of anti-peptide antibodies for the isolation of peptides from a proteolytic digest of a biological sample. The Examiner states that at col. 3, lines 37-47, Little "teach[es] the target polypeptide is isolated prior to being detected by mass spectrometric analysis using a reagent that specifically interacts with the target polypeptide, such as an antibody." Office Action at page 8. The cited passage fails, however, to support the use of anti-peptide antibodies directed against a proteolytic peptide; rather Little merely describes conventional approaches to obtain polypeptides translated in cell free extracts, such as reticulocyte lysates. See col. 3, lines 28-36. Thus, Little describes using antibodies, among other reagents, for isolating proteins from *in vitro* translation reactions, which neither teaches nor suggests contacting **a** sample with a first anti-peptide antibody specific for a first peptide and a second anti-peptide antibody specific for a second peptide, where the second antibody is different from the first antibody.

The passage from Little at column 9, lines 19-25, relied upon in the Office Action also fails to teach the use of anti-peptide antibodies to obtain peptides from a complex proteolytic digest. That passage states the "protein is obtained and subjected to a limited proteolysis prior to mass spectrometric analysis." Thus, proteolysis occurs *after* the protein is obtained, not *before*. Since the steps are carried out in this specified order, there is no suggestion that an affinity method is used on peptides resulting from the digestion. Similarly, the passage at col. 9 lines 26-37 is directed to the isolation of undigested polypeptides, and does not teach or fairly suggest the use of anti-peptide antibodies to obtain proteolytic fragments from a sample after digestion. The distinction between the conventional methods described by Little and the methods recited in the

instant claims is further explained in the Carr supplemental declaration at paragraph 7, which states:

> L ittle describes a conventional use of antibodies for isolation of intact proteins **prior** to digestion by any protease. As such, Little describes isolation of proteins, not peptides, from mixtures that are drastically less complex than those produced by protease digestion.

In addition, neither the passage at col. 9-10, lines 64-5 nor that at col. 6, lines 44-50, teaches or suggests that Little used anti-peptide antibodies to obtain proteolytic peptides from a proteolytic digest. The passage at col. 9-10, merely suggests purifying a polypeptide using a column of solid phase reagent where "the translation reaction is poured over the column." Nowhere does Little teach or suggest that the translation reaction was subject to proteolytic digestion prior to contact with the solid phase reagent or that an anti-peptide antibody was employed. The passage at col. 6, merely defines the nature of the "biological samples" used by Little as the source of DNA coding for the desired polypeptide, and again fails to teach or suggest the use of anti-peptide antibodies to obtain proteolytic peptides from a proteolytic digest.

C. There is no Motivation to Combine the Geng and Little References

In *KSR* the Supreme Court emphasized that an Examiner must provide an explanation to support an obviousness rejection, and that the explanation must include a rational underpinning to support the legal conclusion of obviousness, which cannot be sustained by mere conclusory statements. See also MPEP § 2141 (EXAMINATION GUIDELINES FOR DETERMINING OBVIOUSNESS UNDER 35 U.S.C. 103). Applicant respectfully submits that the Examiner's stated rationale for combining Geng and Little is inconsistent with the admitted failures of the Geng reference and fails to provide a rational basis to support a legal conclusion of obviousness. Accordingly, withdrawal of the rejection respectfully is requested.

In the December 8, Office Action, the Examiner admits that Geng fails to teach the use of anti-peptide antibodies specific for first and second antibodies. Despite this, the Examiner states:

> "It would have been prima facie obvious at the time applicants' invention to modify the method of quantifying an amount of at least a first peptide and a second peptide in a proteolytic digested biological sample by contacting the sample with (i) a first reagent specific for the first peptide and (ii) a known quantity of a labeled version of said first peptide; contacting the sample with (i) a second reagent specific for the second peptide and (ii) a known quantity of a labeled version of said second peptide; separating peptides bound by the first and

> second reagents from unbound peptides; eluting the peptides bound by the first and second reagents; measuring the amount of the first and second eluted peptides using a mass spectrometer; and calculating the amount of the second peptide in the biological sample, as taught by Geng et al., wherein the modification incorporates antibodies in the affinity chromatography techniques as taught by Little et al., because Little et al., teach the need to target and purify the peptide of interest with an antibody that specifically interacts with the target polypeptide prior to being detected by mass spectrometric." (emphasis added)

Simply put, the Examiner alleges that it would have been obvious to replace Geng's lectin affinity reagents with Little's antibody reagents because Little teaches the need to purify a sample prior to mass spectrometric analysis. This rationale fails, however, because it does not address Little's failure to teach or suggest use of anti-peptide antibodies that are specific for proteolytic peptides.

More specifically, the Examiner fails to provide any rationale as to why one of ordinary skill in the art would have been motivated by a reference that describes (1) conventional use of antibodies to obtain proteins from mixtures, followed by (2) protease digestion, to modify a method that uses (1) protease digestion of a mixture followed by (2) use of affinity reagents to obtain *classes* of peptides from the resulting digest.

Moreover, the Examiner's stated rationale ignores the fact that neither reference teaches or suggests using more than one anti-peptide antibody against more than one proteolytic peptide. Geng describes using a single affinity reagent, a lectin, having affinity for glycopeptides, and fails to teach or suggest using two affinity reagents at the same time. Little similarly, fails to teach or suggest using two antibodies at the same time. In either case, using more than one affinity reagent would produce a more complex sample than using a single reagent, which runs counter to the intended use of lectins (Geng) or antibody (Little) to generate a less complex sample for analysis. In the absence of any proper rationale for this modification, no *prima facie* case of obviousness exists and the rejection should be withdrawn.

D. There is No Reasonable Expectation of Success In the Use of Anti-Peptide Antibodies for the Capture of Proteolytic Peptides from a Digest of a Bodily Fluid.

The Examiner further alleges that one of ordinary skill in the art would have had a reasonable expectation of success in combining Geng and Litle. For the reasons set forth above, applicant respectfully submits that no motivation existed to combine the cited references and,

accordingly, there could not, as a matter of logic, have been a reasonable expectation of success in making the combination. Nevertheless, even if a motivation to combine the references somehow were found, there would not have been any reasonable expectation of success.

The Examiner's allegation of a reasonable expectation of success in this instance is unsupported by substantial evidence, and fails to rise to the standard set forth by the Supreme Court in KSR of an explanation supported by a rational underpinning. Specifically, applicant submits that neither of cited references, alone or in combination, or any of the art of record, would have provided a reasonable expectation that anti-peptide antibodies could effectively capture proteolytic peptides from a complex digest resulting from proteolytic treatment of a bodily fluid.

Implicit in the Examiner's allegation in this regard is the assumption that antibodies can be raised against any peptide at will, and that such antibodies would have been expected to be effective in isolating the peptide from a proteolytic digest of a bodily fluid. Applicant respectfully submits that the Examiner's assertions fails to consider the change in the nature and complexity of the sample produced when a bodily fluid is subjected to protease digestion. To address the issues presented when a complex sample is made still more complex by protease digestion, applicant respectfully submits the accompanying Carr supplemental declaration and the Declaration of Professor Terry Pearson ("Pearson declaration"), and also refers the Examiner to the Carr declaration submitted March 10, 2008, in the captioned application.

The Carr and Pearson declarations demonstrate why it was unexpected that proteolytic fragments could be effectively isolated from a complex proteolytic digest by anti-peptide antibodies. Moreover, as indicated in the Pearson declaration, the art provided no reasonable expectation to conclude that it was possible to generate proteolytic peptides that would not only be antigenic and permit generation of antibodies, but that also would be suitable for quantitative mass spectrometric analysis. Furthermore, the ability to raise antibodies against an intact polypeptide/protein does not provide a basis to conclude that antibodies against the native polypeptide/protein would be effective at capturing any of its proteolytic peptides from a complex sample digest.

The Success of the Claimed Methods Was Surprising

The Regnier declaration appended hereto provides still further evidence that the cited references would not have suggested the claimed methods to those of ordinary skill in the art. Professor Regnier is the senior author of the Geng reference, and describes in his declaration how, after hearing details of the methods recited in the instant claims, he approached the applicant and stated that he wished he had thought of the method! Moreover, Professor Regnier goes on to explain not only why the Geng paper did not suggest the instantly claimed methods to him, but also why he does not believe it would reasonably have suggested the claimed methods to a scientist in the field prior to the filing of the instant invention.

The Regnier declaration provides further evidence as to why the Examiner's rationale for the instant rejection is fatally flawed. Accordingly, applicant respectfully submits that no prima facie case of obviousness exists and requests withdrawal of the rejection.

The March 10, 2008 Carr Declaration

The Examiner dismisses the March 10, 2008, Carr declaration on the basis that the cited art allegedly "teaches selection of peptides using specific binding agents, from a complex mixture." That allegation, however, fails to properly assess the statements of the Carr declaration and the teachings of the cited references. The Carr declaration addresses the nature of antibodies as binding agents for peptides and explains why there would have been no expectation that anti-peptide antibodies could be used to select peptides from a complex digest of a bodily fluid. The Examiner's argument that the art teaches the use of specific binding agents from a digested complex serum sample fails to address the relevant issue and conflicts with the Examiner's own admissions with respect to Geng.

Specifically, as the Examiner admits, Geng does not teach the use of antibodies as set forth in the instant claims. Moreover, whatever else Little might teach, it fails to teach or suggest selecting peptides from a complex digest using anti-peptide antibodies. At most, Little appears to describe immunoaffinity capture of translated proteins which may be <u>followed</u> by their digestion to peptides. Nowhere does Little suggest first digesting the sample and then using antibody capture, and nowhere does Little suggest using anti-peptide antibodies that binds proteolytic peptides. Accordingly, the cited references fail to provide any basis upon which the Examiner might dismiss the statements by Dr. Carr related to the capture of peptides using anti-peptide antibodies.

To the extent that the Examiner asserts that the Carr declaration fails to address the nature of the claimed invention, or the scope of the claims, applicant directs the Examiner's attention to the supplemental declaration of Dr. Carr submitted herewith. Applicant further requests the Examiner to provide specific support in the law or rules governing patent practice before the USPTO requiring a declarant to refer to specific claims in order for a declaration to be considered commensurate with the claimed subject matter.

January 14, 2009 email from Professor Randall Nelson

In the Information Disclosure Statement accompanying this response, Applicant includes an email from Professor Randall Nelson received by the undersigned on January 14, 2009 that refers to the response filed August 25, 2008. The paper referred to by Professor Nelson (*Anal. Chem.* **1999,** 71, 2858-2865) also is cited in the Information Disclosure Statement. Professor Nelson's work is discussed in the specification as originally filed for the captioned application and also was discussed in the prior response.

CONCLUSION

In view of the foregoing amendments and remarks, applicants respectfully submit that the application is in condition for allowance. Should the Examiner feel that there are any issues outstanding after consideration of this response; the Examiner is invited to contact the undersigned to expedite prosecution of the application.

The Commissioner is hereby authorized by this paper to charge any fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-3840. **This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).**

Attachments:
Carr supplemental declaration
Regnier Declaration
Pearson Declaration

Date: June 8, 2009

Proskauer Rose LLP
1001 Pennsylvania Avenue, NW
Suite 400
Washington, DC 20004
Telephone: 202.416.6800
Facsimile: 202.416.6899
CUSTOMER NO: 61263

Respectfully submitted,

Joseph W. Ricigliano (Reg. No. 48,511)
Paul M. Booth (Reg. No.: 40,244)

Attorneys for Applicant

Customer No. 61263